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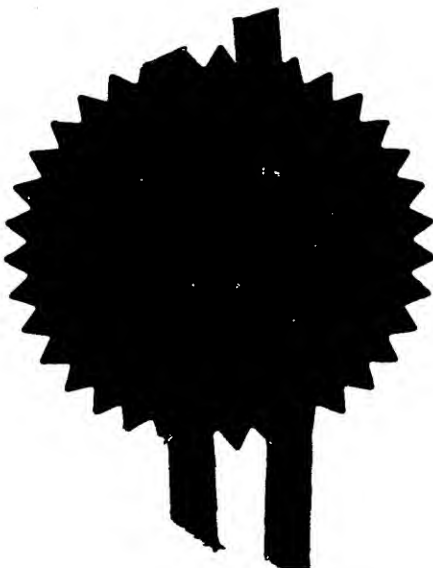
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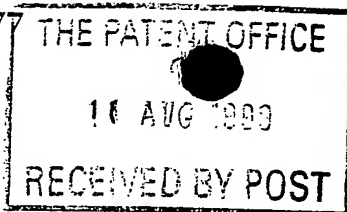
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3. Full name, address and postcode of the or of each applicant (underline all surnames)

The Victoria University of Manchester,
Oxford Road,
Manchester
M13 9PL.

Patents ADP number (if you know it)

If the applicant is a corporate body, give the country/state of its incorporation

773945000
United Kingdom.

4. Title of the invention

SENSOR DEVICES AND ANALYTICAL METHODS FOR THEIR USE.

5. Name of your agent (if you have one)

TUNNICLIFFE, Peter Barry,

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Chester Court,
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Description

12

Claim(s)

Abstract

Drawing(s)

2 + 2

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Translations of priority documents None.

Statement of inventorship and right to grant of a patent (Patents Form 7/77) None.

Request for preliminary examination and search (Patents Form 9/77) None.

Request for substantive examination (Patents Form 10/77) None.

Any other documents (please specify) None.

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Signature

Date 10.08.99.

P.B. Tunnicliffe, Agent for the Applicants. 10 August 1999.

12. Name and daytime telephone number of person to contact in the United Kingdom 01386 858127.

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SENSOR DEVICES AND ANALYTICAL METHODS FOR THEIR USE.

This invention relates to improved sensor devices and methods for their analytical use, and more particularly to improved forms of enzyme electrodes.

There is an increasing need for procedures and devices which can enable the presence and amount of particular components (analytes) in biological media to be measured without having to rely on taking samples periodically and taking them away to be analysed in a laboratory. Such old "sampling" procedures - though usually accurate - are too slow and involve a significant delay in obtaining the result of the measurement, and in many circumstances this delay can be inconvenient and even dangerous to a subject.

Instead, it is very desirable to have a continuing monitoring procedure can be carried and used to detect changes as near as possible to the moment at which they occur, especially if the changes are abrupt or fluctuating. This is important because conditions in some biological environments, and especially conditions in vivo, can change unexpectedly and quickly -- and some measurements may be useful as indications of progress or even critically important as vital indication or warning of a need for speedy remedial action.

Especial interest exists in measuring glucose levels in body tissue or body fluids, as glucose is vital for life and its level is greatly affected by some conditions, for example diabetes mellitus. Other analytes, e.g. drugs and metabolic products, are also of comparable interest.

For a continuous monitoring system, it is very desirable to implant a sensor device in the biological environment or medium, and especially an in vivo environment, so that parameters of a living environment or process can be made. Indeed, the only routinely usable monitoring system for an ambulatory diabetic would be either a portable or an in vivo device.

A variety of such implant devices have been proposed, but their success and applicability vary considerably. The devices or approaches suggested hitherto could, in principle, eventually allow for reliable monitoring but they suffer from various deficiencies and are not entirely satisfactory. For example, many can lack sensitivity or specificity and, as results are often plotted as graphs, show difficulty in making true measurements independently of base-line or response slope variability in such graphs. Further, in some cases it can be impossible to arrive at meaningful in vivo data without in vivo calibration, particularly in a tissue matrix, and the implant may even provoke tissue rejection.

It has been proposed to meet some of these needs by inserting into living tissue a sensor device substantially in the form of a needle incorporating a sensor electrode. Such an electrode may be used in conjunction with a reference electrode - which may be combined with the sensing electrode in the sensor device or may be used separately from it, for example on the skin of the subject.

Such sensors commonly use an active sensor electrode in conjunction with an enzyme, so that the sensor device can be made to respond to selected analyte species which would in themselves be inactive at the electrode and so would not be detected by it, e.g. glucose oxidase for measuring glucose content. Likewise, various forms of coating materials or membranes (permeable or permselective) are commonly proposed for regulating the access of analyte to the active electrode or reducing interference from other compounds which could interfere with the effectiveness of the measurements of the desired analyte if they reach the electrode surface.

It has been proposed to use fine wires of an appropriate electrode material, bare or appropriately coated, compatible with the environment in which it is to be inserted. Even the smallest sensor devices proposed so far have been found to have several difficulties in use, which it would be very desirable to overcome, for example:-

- (1) Present devices can be too large for easy use (e.g. they may cause undue discomfort to the subject) so greater miniaturisation is desirable to reduce the effect the implanted sensor may have on the subject's behaviour and tolerance of the implant. Also it is desirable to achieve a size of device which allows for an adequate balance between rigidity and flexibility, so that the device is more durable and more easily implanted.
- (2) Many known devices employing any coating on the electrode surface - enzyme, membrane, etc. - suffer from difficulties in the stage of insertion into the subject tissue. The enzyme and/or membrane material can be destroyed or displaced using the insertion, to the detriment of the sensor device's effectiveness in subsequent use. This is almost unavoidable, as the friction between the tissue and the coating on the sensor device can be considerable -- especially as the coating material is usually of a very delicate nature.
- (3) It is very difficult to make sensor devices which are both small and effective. The fabrication of very small devices can be difficult, and it is also difficult to achieve the formation of reliable and stable coatings on them. For example, some devices proposed so far are little more than wires having an exposed tip which is the enzyme/membrane system -- and it is especially difficult to coat the tip of a thin wire.

For use, it has been proposed to implant electrodes in tissue through a cannula to avoid undesirable damage during the insertion stage, but this still involves some friction within the cannula. Attempts to enclose the whole device in a sheath of protective material which may contribute to the electrical function of the sensor electrode (e.g. a reference electrode) or as a strengthening or stiffening aid have been proposed, but these tend to increase the size of the device and complicate provision of the necessary degree of exposure of enzyme-coated electrode for effective measuring activity.

The aim of our invention is to reduce the size of sensor implants to the practicable minimum compatible with robust mechanical integrity, and also to provide a compliant insert that reduces patient discomfort beyond that achieved to date.

5 We have now found that miniaturised devices which can overcome such disadvantages can be achieved by modifying the shape of the sensor device (especially one incorporating a wire electrode) by forming cavities in the otherwise smooth material of the sensor device and using these cavities to
10 retain the enzyme. In this way, the enzyme can be retained within the profile of the sensor device and, as it no longer needs to protrude beyond the surface of the sensor device, it is thereby rendered less susceptible to being removed by friction or abrasion during insertion into tissue.

15 Thus according to our invention we provide new sensor devices comprising an enzyme electrode sensor in which active electrode material carries an enzyme, characterised in that the enzyme is retained within one or more cavities formed in the said electrode sensor.

20 By placing the cavity (or cavities) along the length of the electrode core, the enzyme therein can face laterally instead of being on a mechanically vulnerable wire tip.

 The core of active electrode material may be made of any of the conventional conducting materials known for use in the
25 art of sensor electrodes. Preferably it is a noble metal, for example gold or platinum, or an alloy of these with each other or one or more other elements. Preferably the material is platinum itself, but as platinum itself is relatively soft it can be hardened by alloying with a proportion of iridium.

30 The shape of the sensor device is most conveniently of a substantially circular cross-section, as is customarily the case when the active electrode material is a wire core, e.g. conventional drawn metal wire as available commercially, but may be of any other cross-section if desired. The size of
35 the core material is preferably in the range 50 to 150 μm . though larger or smaller sizes may be used if desired. Our

aim is to use as thin a core as can be found to be practical, consistent with the requirement that its strength and integrity are not impaired by the size of the cavities.

5 As the purpose of the construction is for the sensor to present an enzyme-covered surface to its environment, it will normally be found that if any bare active electrode material is also exposed to the environment it will interfere with the measurements made at the surface beneath the enzyme. A bare surface of active electrode material can be tolerated if such
10 interference does not occur, but the preferred form is that in which a core of the active electrode material is covered with a coating of insulating material to prevent bare active electrode material coming into contact with the environment media and the analyte to be detected and measured. Such an
15 insulating material should be suitably durable, stable and resistant to the environment media, effectively sealed over the core of active electrode material, and - when intended for use in vivo - be suitably bio-compatible and harmless in use. Such materials are well known in the art.

20 Consequently, when the sensor to be used has such a coating of insulating material, the cavities required for our invention may be made in several ways. One way is for the insulation to be stripped off to expose a bare core of active electrode material and form the cavity into which the enzyme
25 can then be placed. Alternatively, both the insulation and some of the core of active electrode material can be removed by using an appropriate micro-machining technique, so that the insulation is removed and a cavity is also formed in the core of active electrode material itself. This latter
30 procedure has the advantage that the fabrication is simplified and also that the cavities in the active electrode material allow for a greater surface area of the active electrode material to be exposed to enzyme and used to generate stronger signal outputs.

35 The cavities may be formed by conventional procedures, for example drilling, punching, grinding, boring, cutting, or

any combination of these techniques, and the size and shape of the cavities may take any form which is considered most convenient and capable of retaining the desired enzyme in sufficient quantity. Likewise, the number of cavities may
5 may be as large or small as desired, and will be determined to some degree by the size, shape and position of the cavities used in any particular instance. Our preference is for the cavities to be of a size up to about half of the overall thickness of the sensor material, so that the
10 strength of the sensor is not unduly reduced. The optimum in any particular case may be readily determined by simple trial. The method used for forming the cavities may be a mechanical one, though that can be difficult on the micro-scale required; therefore we prefer to use an ion beam or
15 laser method (commonly referred to a "micro-machining") as this is more easily used on the scale of size involved here. Thus a laser or ion beam can be used to etch, cut or bore into the material of the sensor to form the required cavities.

20 Examples of suitable shapes for the cavities include circular, oval, square, polygonal, cruciform, star-shaped and combinations of these. The cavities may regular or irregular in their, size, shape, number and distribution, though it is generally preferred (as being more convenient) to make all
25 the cavities of substantially the same shape and size. It is preferred that the form of the cavities should be chosen so that they can readily retain the enzyme; thus dish-shaped cavities may be less efficient if the enzyme is not strongly held, and cavities which are more like "pits" are usually to
30 be preferred as they achieve a stronger hold on their enzyme contents.

Another useful cavity shape is a slot cut into the sensor in a substantially lengthways direction (i.e. in the direction of the axis of a wire electrode), as this can
35 minimise the number of cavities to be made. The size of the slot (length and breadth) may be varied to suit particular

needs and usually are not critical.

5 An especially useful form of cavity is one which passes completely through the core of electrode material, in effect forming a tunnel, open at both ends, running transversely to the general direction of the inner core. This allows the enzyme to be packed into this tunnel and the enzyme contents to be exposed to analyte and (in the case of an oxidase) also oxygen, as needed for reaction - thus giving very effective enzymatic action and consequent measurement efficiency. If
10 desired the cavity may contain more than one enzyme, e.g. as laminate layers, so that a succession of reactions can be catalysed --- one enzyme acting on an analyte substrate to form a product which, in turn, is acted upon by the second enzyme to generate a further product which can then be
15 satisfactorily detected and measured at the active electrode surface.

The enzyme may be used in conventional formulations and compositions, and placed in the cavities and retained therein by conventional coating methods and fixing methods. For
20 example the enzyme may be applied as a composition which coats and fills the cavities (e.g. by dipping) followed by wiping or passing through a collar to remove the surplus (and especially any on the main surface where it is not required. The enzyme can then be fixed in place by cross-linking, e.g.
25 by treatment with glutaraldehyde.

The enzyme may be any of the conventional enzymes used in sensor enzyme electrodes for electrochemical analysis, but we find oxidase or dehydrogenase enzymes are most useful. An especially useful example is glucose oxidase, which allows
30 the device to be used for detection and measurement of glucose concentrations in tissues. Though we describe our invention with particular reference to glucose and glucose oxidase, however, it is not limited to this specific system and it is applicable to other substrate/enzyme systems, of
35 which several are well known in the art for analytical purposes.

The sensor devices of our invention preferably also comprise coatings over the enzymes held within the cavities. Thus, additional layers of material may be deposited over the enzyme after that has been put into the cavities. Such over-
5 coating layers may be composed of materials of appropriate permeability (simple or selective) to regulate the passage of components from a sample under examination to the enzyme and active electrode surface, or excluding or limiting access by materials which could interfere with the measurements. These
10 materials are well known in the art and are usually in a thin form which serves as a permselective membrane, and may be applied by conventional means also well known in the art.

Examples of such materials include various polymers and polymer compositions, e.g. polyaryl ether sulphones and
15 modified polyurethanes.

The electrodes of this invention may be used by any of the conventional procedures well known in the art, but of all the electrochemical procedures available we prefer to use an amperometric procedure with the active electrode material as
20 the anode.

The sensor devices of our invention can be used in vivo or in vitro, and the mode of insertion into the sites for making measurements are conventional. For in vivo sites, they may be inserted directly (transcutaneously into tissue)
25 or through a cannula or even fine tubing. e.g. of nylon.

The advantages of the invention are especially in the way it provides new sensors which are small, light in weight, and potentially much more robust, flexible and suitable for implantation without attendant discomfort and other problems.
30 Other advantages stem from the way in which the new sensors provide a different orientation and profile for the sensing surfaces - side-oriented micro-machined sensing surfaces - to the samples under examination,

The invention is illustrated but not limited by the
35 accompanying drawings, which illustrate some forms which the improved electrodes can take.

These drawings are schematic and not drawn to scale, but are intended to show the principal features - in some cases emphasised by being out of scale.

In the drawings Figures 1 to 4 represent, in perspective view, various forms of sensor electrode devices of according to this invention and the type and disposition of cavities, and Figures 5 to 10 illustrate cross-sectional views through such sensor devices at the position at which the a cavity is made.

In detail, all of Figures 1 to 4 show a thin platinum wire covered with insulation (1), with the said insulation covering the end (2) as well as the main body (1). The end (3) is adapted for continuation on and connection to the electrical measuring system (not shown).

In Figure 1, which shows the simplest form of the invention, the insulated wire (1) is pierced by a hole (4) to form a cavity (5) which is filled with an immobilised enzyme composition.

In Figure 2, there is shown a form in which the insulated wire (1) is pierced by a series of holes (6), each forming cavities filled with immobilised enzyme as in Figure 1. In Figure 3, there is shown a form in which the insulated wire (1) is pierced by a series of holes (7), each forming cavities filled with immobilised enzyme as in Figure 1 but of a shape different from those in Figure 2, i.e. cruciform instead of round.

In Figure 4, there is shown a form in which the insulated wire (1) is stripped of its insulation to form a slot (8) in the direction of the axis of the insulated wire (1), and the resulting slot is filled with immobilised enzyme; in this variant, the slot (8) has been made by cutting out the cover layer of insulation but without cutting into the core of platinum wire itself, but a further alternative (not shown) is that of cutting the slot into the core of platinum metal in addition to cutting away the outer layer of insulation.

All of Figures 5 to 10 illustrate cross-sectional views of alternative forms of the cavity through a thin platinum wire covered with insulation where the cavity is made.

In Figure 5, which shows the simplest form of the invention, the insulated wire comprises a core of thin platinum wire (1) covered by an outer layer of insulation (2) with a part of the insulation cut away to leave a cavity (3) filled with an immobilised enzyme composition.

In Figure 6, a cavity (3) has been bored through the inner platinum wire core, passing through it completely from one side to the other, and is filled with an immobilised enzyme composition. The enzyme-filled cavity (3) is coated at each end with a layer of a membrane coating (4) which acts to protect the enzyme and provide a chosen degree of selectivity or regulation of access of components of the surrounding medium to the enzyme.

In Figure 7, the cavity (3) has been bored through the inner platinum wire core, passing through it completely from one side to the other, and is filled with an immobilised enzyme composition as in Figure 6, except that two different enzyme compositions (3A) and (3B) are used to fill the cavity (3). The enzyme contents are coated at each end with a layer of a membrane coating (4) as in Figure 6.

In Figure 8, the arrangement is essentially the same as for Figure 5 except that the cavity (3) is bored through the outer insulation layer (2) and also into the inner platinum wire core to form an inner pit in the platinum. This form has the advantage of having a larger surface area of the platinum exposed to enzyme and this produces greater response signals for measurement.

In Figure 9, the arrangement is essentially the same as for Figure 6 except that the cavity (3) bored through the outer insulation layer (2) and completely through the inner platinum wire core has not been given any coating (4), so that the enzyme composition contents of the cavity (3) are exposed directly to the surrounding media.

In Figure 10, the construction is that of Figure 6 but shows - with added arrows (9) - indication of flows of fluid past the membrane-covered apertures in the insulation. These flows may be fast or slow, large or small, and may be the same or different. One variant which is practicable is for the two flows (9A) and (9B) to be different -- with one (9B) being the sample medium, from which analyte components can diffuse in to the enzyme through the membrane, while the other flow (9B) can provide similar access by diffusion for necessary substrates for the enzyme system. For example, (9A) may provide the main source of glucose to a glucose oxidase system while (9B) may provide more of the oxygen necessary for the enzyme to function.

To fabricate the devices illustrated, the insulated platinum wire may be etched to remove the insulation layer and expose the platinum within, and subsequent polymerisation or other conventional techniques may be used to deposit the enzyme on the platinum surface. Covering the enzyme in the cavity can be achieved by conventional techniques, for example application from solution by dipping. The thickness of the coating may vary, but should be thin enough to allow adequate diffusion of a desired analyte without unduly impeding the ability of the material contacting the enzyme to change frequently enough to give a satisfactory overall view of the rate of changes there may be in the medium surrounding the sensor device.

The etching and boring operations indicated above were carried out using a copper laser.

The thin platinum wire used is a commercially available one, as commonly used for making wire electrodes, since it is not necessary to use a special wire. If the coating of insulation on commercially available wire is not suitable for use in any particular intended environment, the coating as supplied can if necessary be removed and replaced by re-coating with a more suitable insulation or a coating of the preferred insulation material may be deposited over the one already on the commercial wire.

The method of fabrication according to this invention, and illustrated herein, enables insulated monopolar platinum wire electrodes having an outer diameter of 50 to 150 μm and a series of laser-drilled transaxial cavities (fenestrations) each approximately 30 μm in diameter for wire-shaft enzyme loading to be made without undue difficulty.

Using laser-etching of an insulated platinum wire of 25 to 50 μm outer diameter to remove insulation locally allows further miniaturisation and can create an ultra-fine working electrode surface on which thin layers of enzyme and barrier films can be deposited.

These provide embodiments provide protection for the active enzyme and have unique device geometry; configurations as illustrated provide side-oriented enzyme surfaces in micro-machined sensing surfaces designed to retain and protect them.

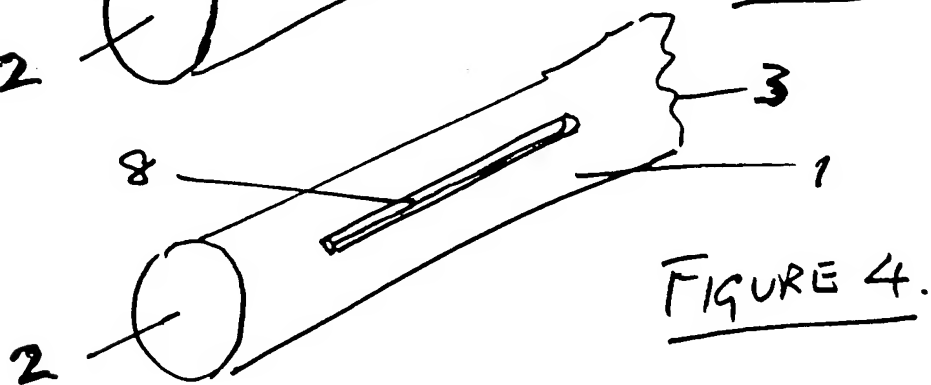
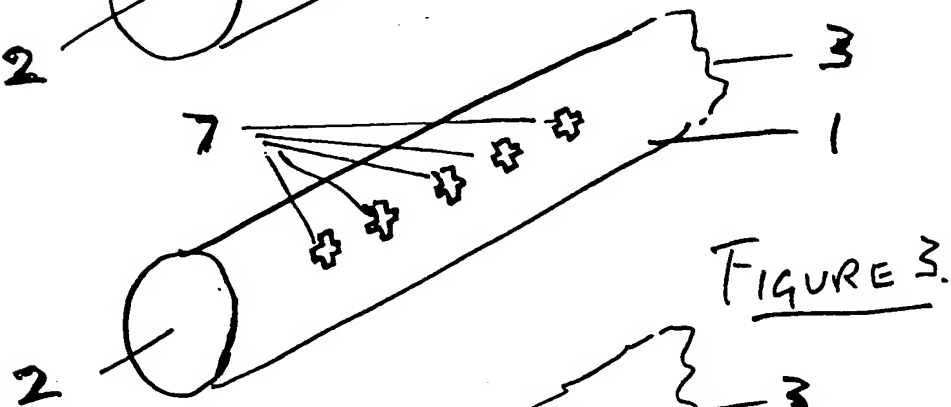
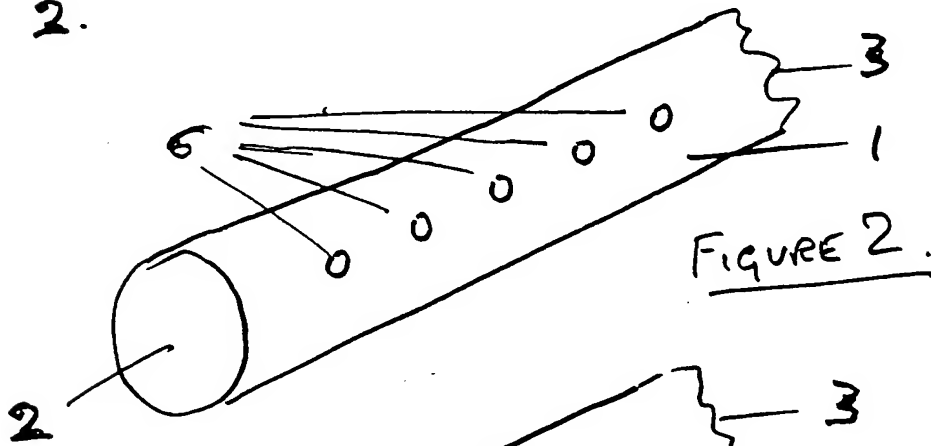
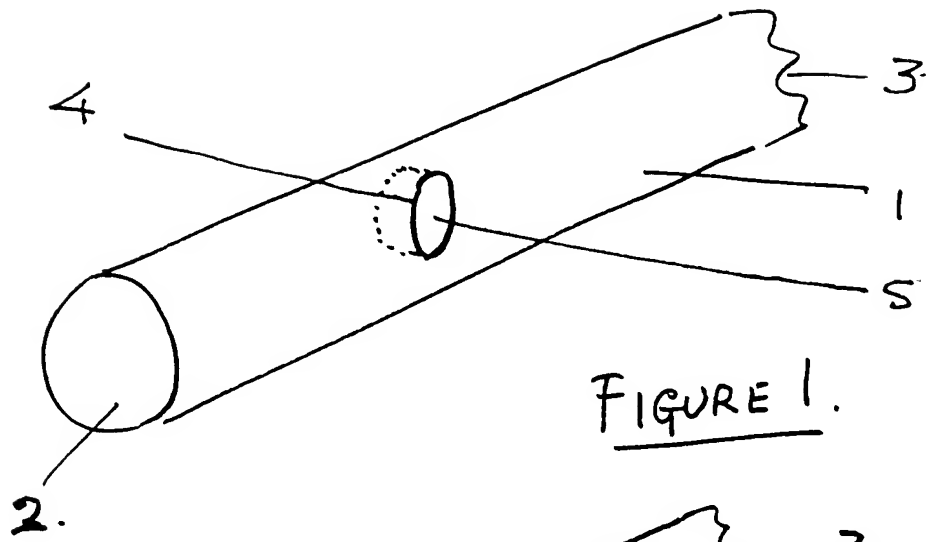
Construction as in Figure 6 in particular internalises the enzyme in the working electrode, giving a unique twin-surface enzyme with radial product diffusion into the surrounding platinum surface. The greater area of active platinum surface (i.e. surface exposed to the enzyme) can improve the efficiency with which the enzyme functions and so improve the measurements, for example in the linearity of response.

In use, the device can be inserted into tissue and, though the tip naturally must enter first, this entry does not damage the enzyme-containing areas of the surface. When the wire is pure platinum it may be too soft for direct insertion to be easy, so subcutaneous implantation of such "soft" wires will be through narrow bore nylon tubes. Alternatively, direct insertion can be made easier by using a platinum/iridium alloy wire, which is more rigid.

For determination of analytes, the procedures used were conventional ones and not described in detail here as they are well known in the art and do not involve any departures from the usual ones.

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P. 444.



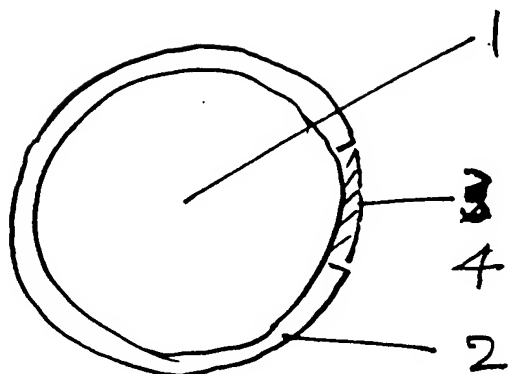


FIG. 5.

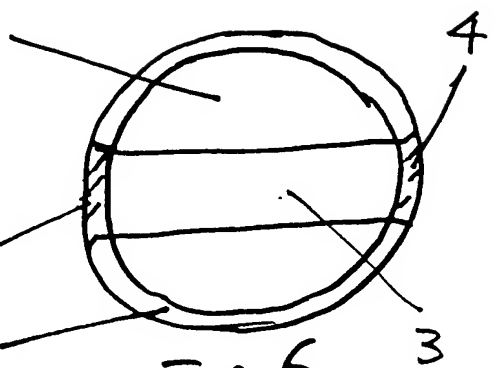


FIG. 6.

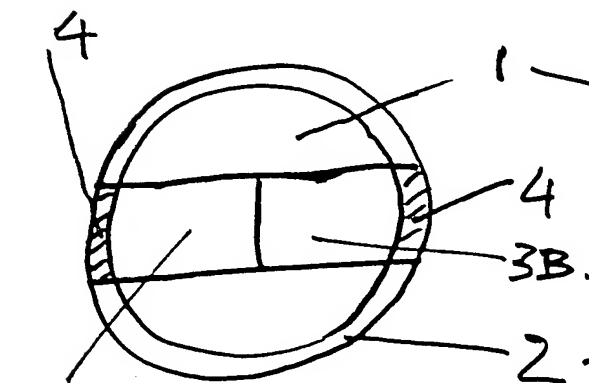


FIG. 7.

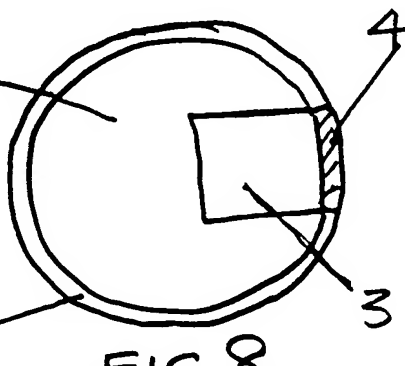


FIG. 8.

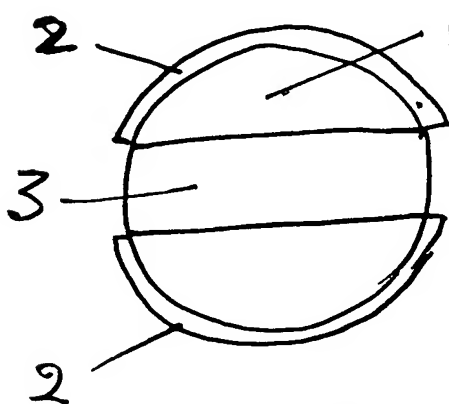


FIG. 9.

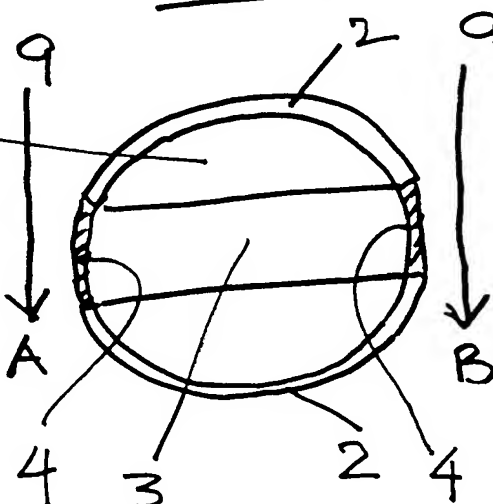


FIG. 10.

100